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237. Motoji Asai, Harukiyo Hieda, and Bunji Shimizu*1 : Studies on Synthetic Nucleotides. IV. Preparation of L-β-Ribonucleosides and L-β-Ribonucleotides.*2

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The chloromercuri salt of 6-benzamidopurine was condensed with 2,3,5-tri-O-benzoyl-DL-ribofuranosyl chloride, then the protecting groups removed, β-DL-adenosine was produced. β-DL-Adenosine was treated with nitrous acid to afford DL-inosine. β-DL-AMP was obtained by phosphorylation of β-DL-adenosine with phenylphosphorodichloridate. In a similar manner β-DL-IMP was obtained starting from β-DL-inosine.

Attempts were made with success to obtain L-adenosine by incubation of DL-adenosine with a bacterial cell suspension (the strain used was PS-264, an unidentified stock culture of our laboratories). The incubation for 2~3 days resulted in complete deamination and hydrolysis of D-adenosine whereas about 50% of L-adenosine remained unattacked and the rest was deaminated to give the corresponding L-inosine.

On the other hand, when DL-AMP was incubated with a snake venom obtained from *Trimeresurus flavoviridis*, only D-antipode was dephosphorylated to D-adenosine and L-AMP left unchanged. L-AMP was separated from D-adenosine by ion exchange chromatography. L-IMP was also obtained from DL-IMP by the same method.

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The sugars found in natural nucleic acids are D-ribose and D-deoxyribose in almost all cases.

D-Ribose is also present in the nucleotide coenzymes such as nicotinamide-adenine dinucleotide (NAD), flavin-adenine dinucleotide (FAD) and vitamin B_{12} as well as antibiotics such as tubercidin[1] and toyocamycin.[2] Although previous investigations have exclusively been concerned with chemical and biological studies of the D-isomers of ribonucleosides and ribonucleotides, there have been no reports of biological studies of the corresponding L-isomers accessible only by chemical methods. Also neither nucleosides nor nucleotides having L-ribose have been known, except L-adenosine, recently synthesized by Goodman, *et al.*[3]

Interest in the biological activity of nucleoside and nucleotide analogs led us to investigate the synthesis of L-β-derivatives of these compounds which might act as antimetabolites or anticancer agents. This paper reports the successful preparation of the new L-derivatives involving the optical resolution of DL-isomers of nucleosides and nucleotides prepared from DL-ribose[4] with the aid of micro-organism and enzymes.

The authors report two methods for the synthesis of nucleotides. One[5] is a method involving silylation of purine and pyrimidine bases followed by fusion with phosphorylated sugar halides (Chart 1). The other method[6] involves heating the bases and phosphorylated sugar halides in the presence of polar organic solvents.

These methods are especially favorable, for purine nucleotides. The desired nucleotides have been obtained by de-phenylation of the final product, nucleoside

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*2 Brief reports of a part of this work were published as a communication to the Editor in This Bulletin, **13**, 616 (1965).

1) S. Suzuki, S. Marumo : J. Antibiotics (Japan), Ser. A, **13**, 360 (1960).
2) K. Ohkuma : *Ibid.*, **13**, 361 (1960).
3) E.M. Acton, K.J. Ryan, L. Goodman : J. Am. Chem. Soc., **86**, 5353 (1964).
4) I. Iwai, T. Iwashige, M. Asai, K. Tomita, T. Hiraoka, J. Ide : This Bulletin, **11**, 188 (1963).
5) B. Shimizu, M. Asai, T. Nishimura : *Ibid.*, **15**, 1847 (1967).
6) M. Asai, M. Miyaki, B. Shimizu : *Ibid.*, **15**, 1856 (1967).

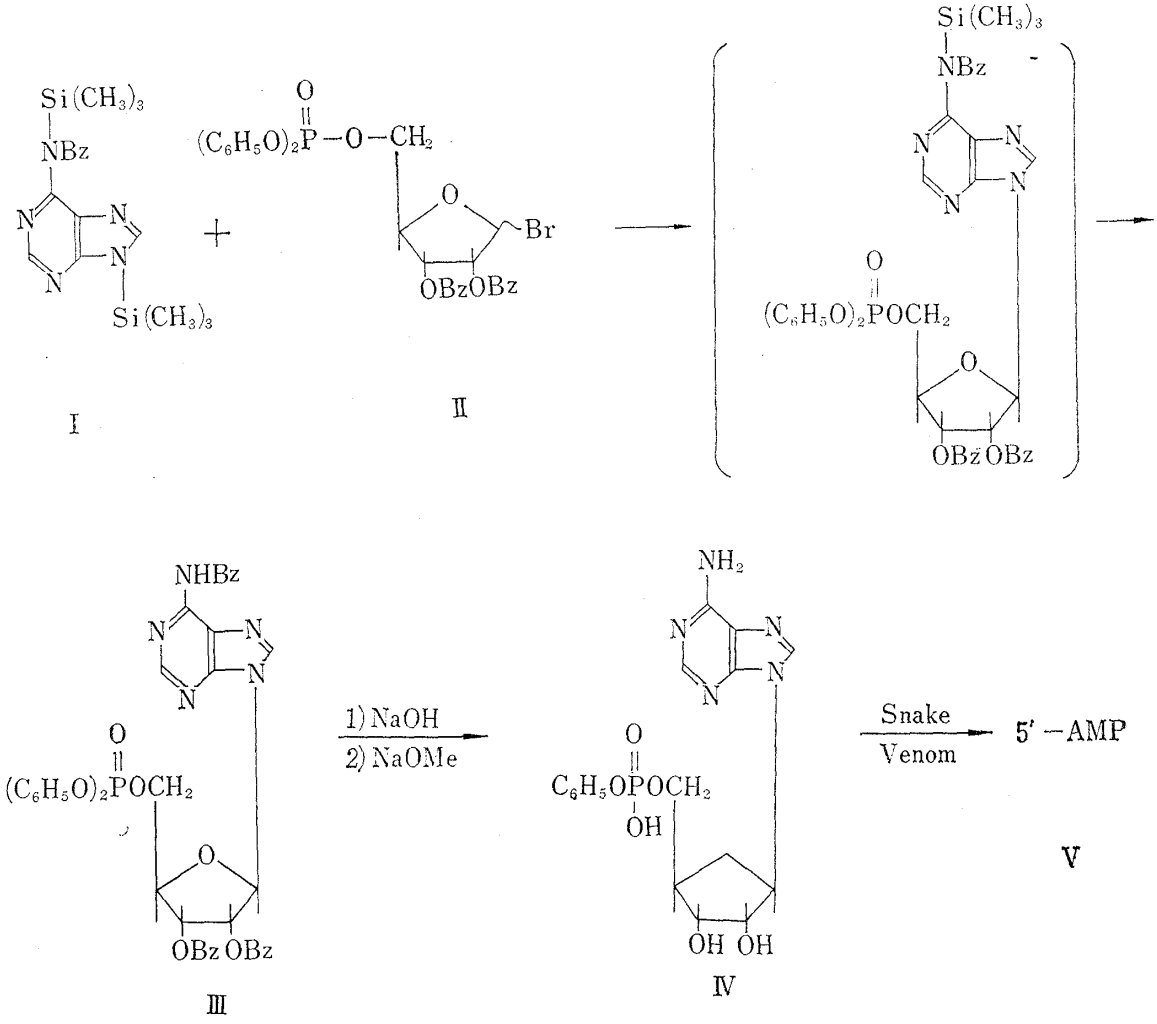

Chart 1.

5′-phenylhydrogen phosphate, with phosphodiesterase,[5] prepared from *Trimeresurus flavoviridis* (Hallowell). However, in the case of the DL-series, the ratio of components D to L in the nucleotide formed may be unequal because of the presence of 5′-nucleotidase in the enzyme, the racemate not being produced. Therefore, it is necessary to produce the nucleotide by chemical methods.

As it is the principal objective of this investigation to prepare β-DL- and β-L-nucleosides, the synthesis of nucleosides was done by the chloromercuric method. Synthesis of nucleotides is carried out using phosphorylating agents with which removal of the protecting groups is easy. According to the method of Baker, *et al.*[7] 1-acetyl-2,3,5-tri-O-benzoyl-DL-ribofuranose (Ⅵ) prepared from DL-ribose[4] was converted to the chloride by conventional procedures using hydrochloric acid in ether. The chloride was condensed with chloromercuric-6-benzamidopurine by the method of Davoll,[8] followed by removal of the protecting groups to give DL-adenosine (Ⅶ); the latter was converted to the isopropylidene derivative (Ⅷ), which was then phosphorylated with phenyl phosphorodichloridate according to the method of Ikehara, *et al.*[9] After removal of the protecting groups, the crude nucleotides formed were separated by column chromatography on carbon powders,[10] to give DL-adenosine 5′-phosphate (DL-AMP).

7) H.M. Kissman, C. Pidacks, B.R. Baker: J. Am. Chem. Soc., **77**, 18 (1955).
8) J. Davoll, B.A. Lowy: *Ibid.*, **73**, 1650 (1951).
9) M. Ikehara, E. Ohtsuka, F. Ishikawa: This Bulletin, **9**, 173 (1961).
10) Y. Sanno, M. Honjo, K. Tanaka: *Ibid.*, **9**, 657 (1961).

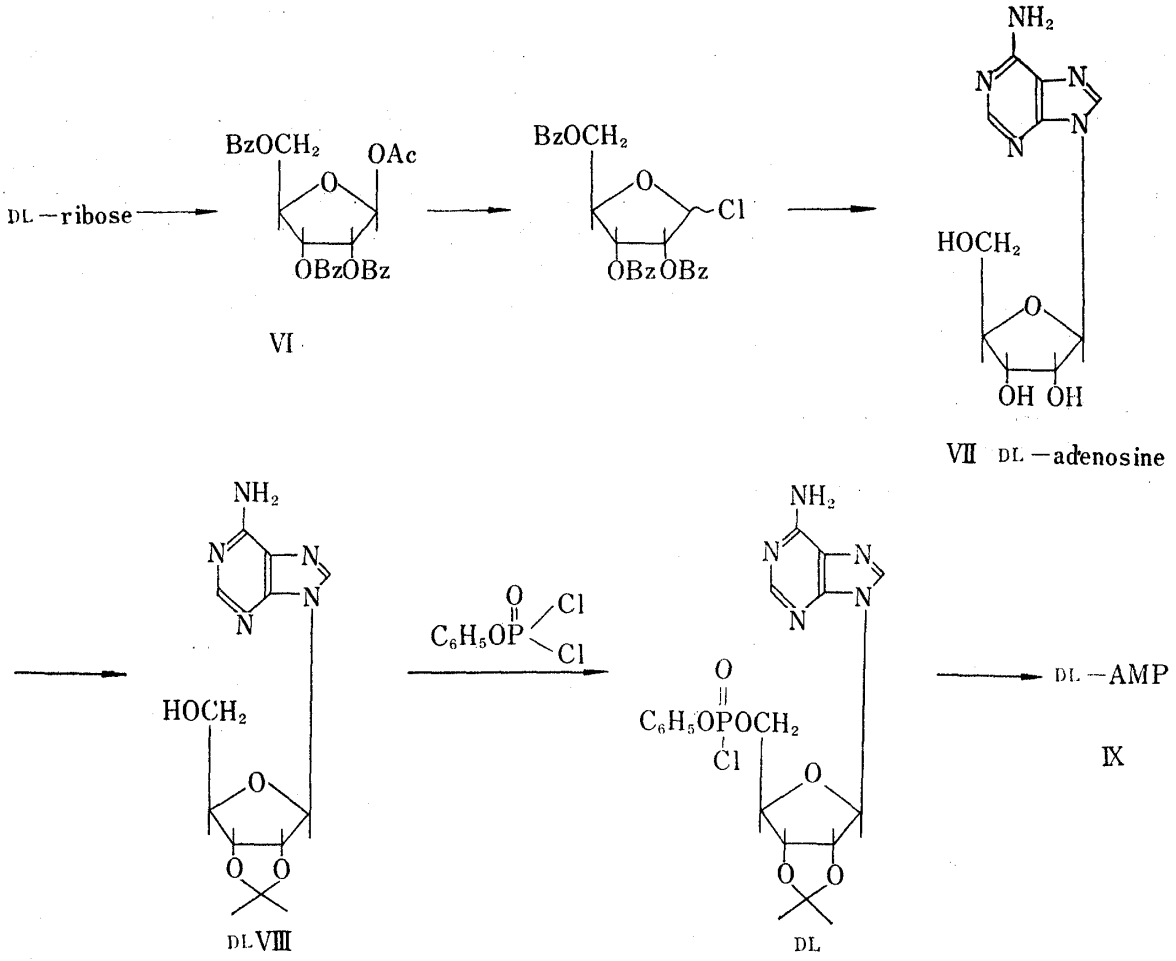

Chart 2.

By diazotation, DL-adenosine (VII) was converted to DL-inosine (X), which was in turn converted to the isopropylidene derivative (XI), followed by phosphorylation with tetra-*p*-nitrophenyl pyrophosphate[11] and removal of the protecting groups to give DL-inosine 5′-phosphate (DL-IMP) (Chart 3).

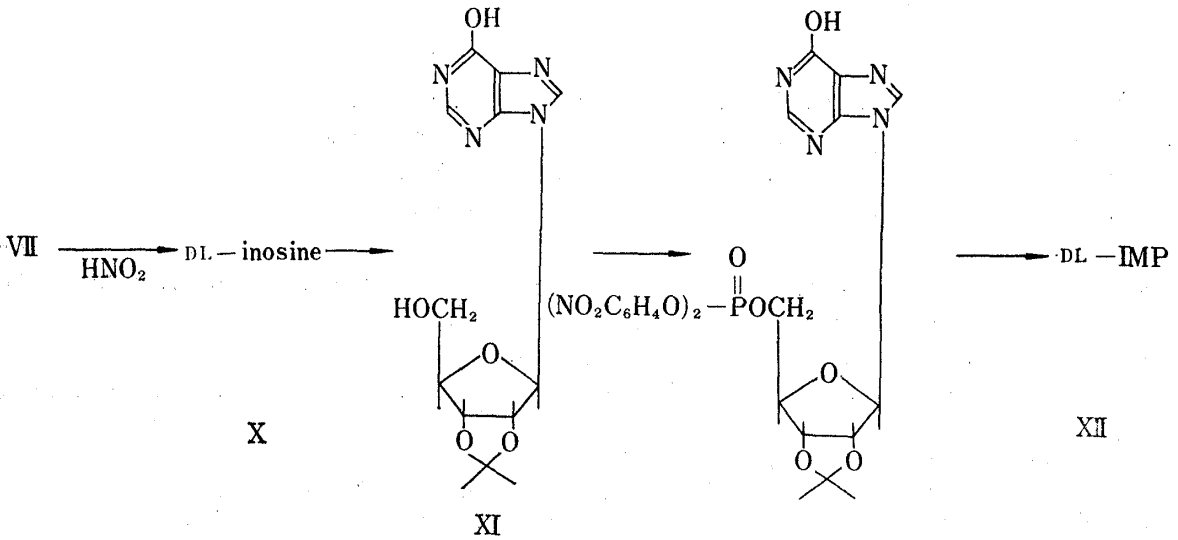

Chart 3.

11) J.G. Moffatt, H.G. Khorana : J. Am. Chem. Soc., **79**, 3741 (1957).

Chemical[12,13] and microbiological[14] processes have been known for the optical resolution of sugars, but no report is found in the fields of nucleosides and nucleotides. The authors attempted the resolution of racemic nucleosides.

Enzymatic phosphorylation of nucleosides[15~17] was attempted in view of the possible separation of the L-isomer in the form of the original nucleoside from only phosphorylated D-nucleotide. The attempt failed at the preliminary stage because of the presence of D-nucleoside remaining due to incomplete phosphorylation of the D-isomer. Next, an attempt was made to separate the D-nucleoside. In the preliminary experiments microorganisms were screened for decomposition of D-adenosine, with L-β-adenosine remaining unchanged. Among these a strain PS-264 (in the culture of this laboratories, *Pseudomonas Ovalis*) was selected for this purpose.

D-Adenosine was incubated with a cell suspension of the PS-264; after 24 hours hypoxanthine began to be formed and after 48 hours, no D-adenosine was recognized. There was no material then detected on paper chromatography (Fig. 1~3).

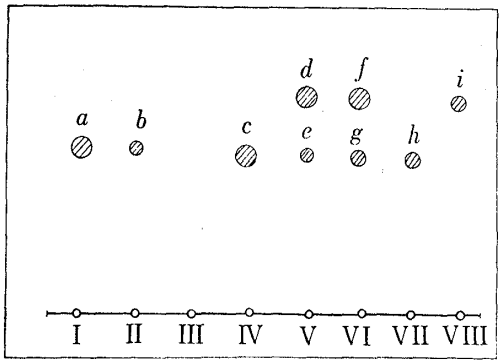

Fig. 1. Paper Partition Chromatograms of the Incubation Mixture of D- and DL-Adenosine and Bacterial Cell Suspension (PS-264 strain)
Solvent : water at 10°

a : D-adenosine
hb : hypoxanthine
c : DL-adenosine
fd : L-inosine
eg : L-adenosine
i : D-inosine

	Substrate	Incubation time (hr.)
I	D-adenosine	0
II	D-adenosine	24
III	D-adenosine	48
IV	DL-adenosine	0
V	DL-adenosine	48
VI	DL-adenosine	120
VII	hypoxanthine	0
VIII	D-inosine	0

Then, DL-adenosine treated in the same way gave two spots on paper chromatography, after enzymatic decomposition of the D-isomer for 48 hours. The same result was obtained after 120 hours of enzymatic decomposition (Fig. 1,4). These substances were subjected to isolation by means of paper-chromatography or ion-exchange (Dowex-1 in $HCOO^-$ form) chromatography. One of the separated crystals melted at 218° and were identical with inosine on the basis of UV, Rf and electrophoretic characteristics. $[\alpha]_D$ was +48.7°, which was equal in absolute value but reverse in sign to the natural D-isomer. The crystals were thus identified as L-inosine. The other crystals melting at 230° were identical with adenosine in physical constants. As was the case with the

12) C. Neuberg, M. Federer : Ber., **38**, 866, 868 (1905).
13) R.B. Woodward, T.P. Kohman, G.C. Harris : J. Am. Chem. Soc., **63**, 120 (1941).
14) G.R. Noggle : The Carbohydrates (Chemistry, Biochemistry, Physiology) Ed. by W. Pigman, 619 (1957), Academic Press, Inc., New York.
15) H. Katagiri, H. Yamada, K. Mitsugi, M. Takahashi : Agr. Biol. Chem., **27**, 469 (1963); **28**, 577 (1964).
16) K. Mitsugi, K. Komagata, M. Takahashi, H. Iizuka, H. Katagiri : *Ibid.*, **28**, 586 (1964).
17) K. Mitsugi : *Ibid.*, **68**, 659, 669 (1964).

former product $[\alpha]_D^{27}$ +59.4° indicated that the latter was the antipode of D-adenosine, that is, L-adenosine.

D-Adenosine was completely converted to D-inosine on reacting for 1 hour with a deaminase separated and purified from a strain of *Aspergillus oryzae*, by Minato[18] of this laboratory whereas L-adenosine was deaminated at this stage only to a very small extent. After 24 hours, however, L-inosine was produced but some L-adenosine was found, unchanged. A new resolution of racemic nucleotides was attempted using 5′-nucleotidase[19] obtained from *Trimeresurus flavoviridis* (Hallowell), with which dephosphorylation of only the D-isomer was

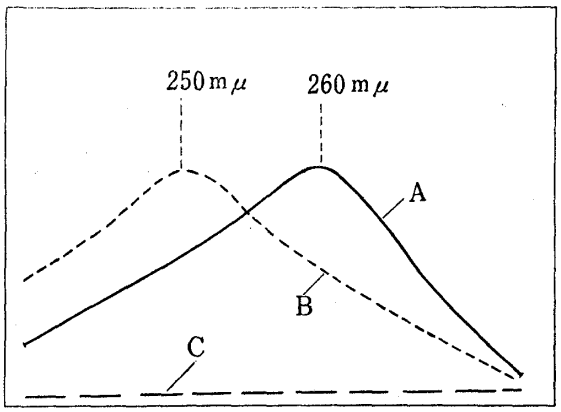

Fig. 2. Ultraviolet Absorption Spectra of the Incubation Mixture of D-Adenosine and Bacterial Cell Suspension (PS-264 strain) at pH 7.0 and 30°
(A) initial: (B) after 24 hr., (C) after 48 hr.

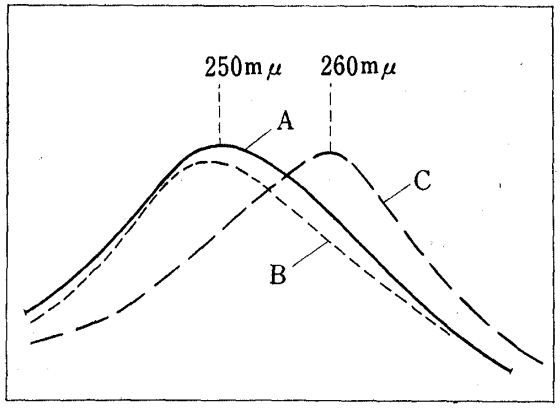

Fig. 3. Ultraviolet Absorption Spectra of the Degradation Product of D-Adenosine by Bacterial Cell Suspension (PS 264 strain) after 24 hr. at Different pH Values
(A) pH 7: (B) pH 1, (C) pH 13.

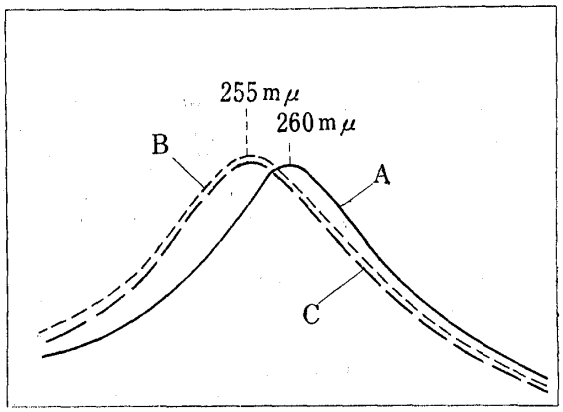

Fig. 4. Ultraviolet Absorption Spectra of the Incubation Mixture of DL-Adenosine and Bacterial Cell Suspension at pH 7.0 and 30°
(A) initial: (B) after 48 hr., (C) after 120 hr.

possibly feasible in view of its substrate specificity, to give the L-nucleotide and D-nucleoside. In the preliminary experiment, D-adenosine 5′-phosphate was used for the enzymatic decomposition, in which D-adenosine was produced in 2 hours.

Reaction of DL-AMP with the enzyme for 2.5 hours gave two spots on paper chromatography. The two were resolved by ion-exchange chromatography on Dowex-1, in $HCOO^-$ form. One was adenosine, $[\alpha]_D^{24}$ −60° being identical with that of the natural D-isomer. The other was indicated to be AMP in physical properties except that the optical rotation, $[\alpha]_D^{26}$ +37°, was equal in absolute value but reverse in sign to the natural one. It was evidently L-AMP. The same applied to the action of 5′-nucleotidase on D-inosine 5′-phosphate (D-IMP) and DL-IMP, from which D-inosine and L-IMP as well as D-inosine were obtained respectively. It follows as expected that 5′-nucleotidase has substrate specificity for the DL-compounds.

18) S. Minato, T. Tagawa, M. Miyaki, B. Shimizu, K. Nakanishi: J. Biochem. (Tokyo), **59**, 265 (1966).
19) The enzyme preparation was obtained through the courtesy of Dr. K. Ikezawa of the National Institute of Health, Japan.

To sum up, total synthesis of L-β-adenosine, L-β-inosine, L-AMP and L-IMP has been successfully carried out by the authors (Chart 4). In assessing the biological activity of L-adenosine itself in a suitable mammalian cell system, it was observed[20] that L-adenosine was unable to replace D-adenosine to support the growth of mammalian cell cultures in a system requiring an exogeneous purine source for optimal growth, but this L-nucleoside was found to be non-toxic. Biological studies of the other L-derivatives prepared in our laboratories are in progress.

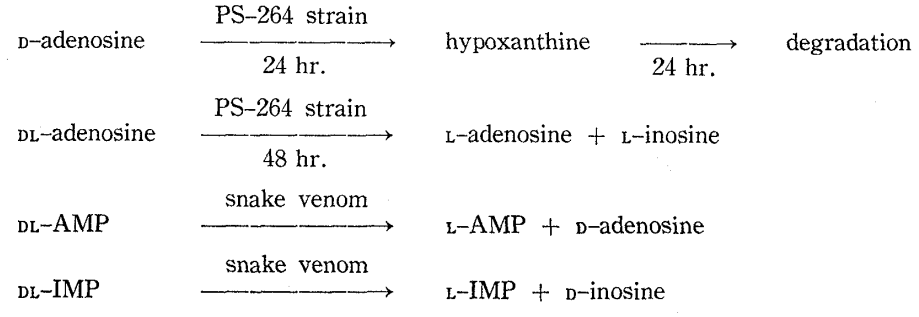

Chart 4.

Experimental

1-Acetyl-2,3,5-tri-O-benzoyl-β-DL-ribofuranose (VI)——1-Acetyl-2,3,5-tri-O-benzoyl-β-DL-ribofuranose (VI) was obtained from 30 g. of DL-ribose following the method by Baker, *et al.*[7] The crude product (VI) was recrystallized from dil. MeOH as crystals (52.5 g.), m.p. 118~119°. *Anal.* Calcd. for $C_{38}H_{24}O_9$: C, 66.66; H, 4.80. Found : C, 66.75; H, 4.68. Compound VI was identified with an authentic 1-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose by IR spectrum.

β-DL-Adenosine (VII)——β-DL-Adenosine (VII) was prepared from VI following the method by Davoll.[8] 2,3,5-tri-O-Benzoyl-DL-ribofuranosyl chloride prepared from 5.0 g. of VI and ethereal HCl, and 3.8 g. of chloromercuri 6-benzamidopurine was reacted on celite in dry xylene. 6-Benzamido-9-(2,3,5-tri-O-benzoyl-β-DL-ribofuranosyl)purine thus obtained was deprotected with cold solution of ammonia in MeOH (saturated at 0°). Concentration of the solvent *in vacuo* gave a solid (VII) which was recrystallized from dil. MeOH as crystals (1.9 g.), m.p. 245° (decomp.). *Anal.* Calcd. for $C_{10}H_{13}O_4N_5$: C, 44.94; H, 4.90; N, 26.21. Found: C, 44.77; H, 5.06; N, 25.74.

2′,3′-Isopropylidene-β-DL-adenosine (VIII)——A mixture of 1.0 g. of VII, 7.0 ml. of dry acetone, 3.5 ml. of ethylorthoformate and 10 ml. of ethanolic solution containing 0.7 g. of HCl was shaken to dissolve VII. After a few minutes VIII precipitated. The reaction mixture was neutralized with methanolic ammonia at −10~−5°, and was evaporated to dryness *in vacuo*. A few milliliters of water were added to the residue and insoluble VIII was collected and recrystallized from dil. acetone as crystals (0.9 g.), m.p. 207~208°. *Anal.* Calcd. for $C_{13}H_{17}O_9N_5$: C, 50.81; H, 5.58; N, 22.79. Found : C, 50.53; H, 5.49; N, 22.82. Compound VIII was identified with authentic 2′,3′-isopropylidene D-adenosine by IR spectrum.

β-DL-Adenosine 5′-Phosphate (DL-AMP) (IX)——To a solution of 450 mg. of VIII and 188 mg. of quinoline in 8 ml. of dry dioxane was added slowly at 10~12° 340 mg. of phenylphosphorodichloridate in 4 ml. of dry dioxane. From the reaction mixture isopropylidene-β-DL-adenosine 5′-phosphate was obtained following the procedure reported by Ikehara, *et al.*[9] The isopropylidene derivative was heated with aq. solution (pH 1.5) at 80~85° for 1 hr. giving the impure nucleotide (IX). This was absorbed on active carbon and was thoroughly washed with water. The nucleotide was eluted with NH_4OH (1.5% solution). All fractions were checked by UV spectrometer and fractions containing IX were combined, and evaporated to dryness *in vacuo* giving the ammonium salt of DL-AMP (100 mg.). This was dissolved in water and Dowex 50 (H^+) was added to the solution to adjust the pH to 2.0. The aqueous solution was evaporated to dryness *in vacuo* affording DL-AMP (IX). Compound IX was identified with natural 5′-AMP by UV, Rf value and electrophoresis.

DL-Inosine (X)——DL-Adenosine (VII, 1.65 g.) was dissolved in 125 ml. of hot water and cooled. To the solution was added 3.3 g. of $NaNO_2$ in 4.1 ml. of AcOH and the mixture was set aside overnight at room temperature. The solvent was evaporated to dryness *in vacuo* and if necessary, to the residue was added a few milliliters of EtOH and evaporated *in vacuo* for removal of a trace amount of water. The residue was refluxed with a mixture of 6 ml. of Ac_2O and 5 ml. of dry pyridine for 30 min. The reaction mixture was evaporated to dryness *in vacuo* and the residue was dissolved in $CHCl_3$. After separating an insoluble

20) K. Ohnishi : Unpublished data.

inorganic salt, the solution was evaporated to dryness. Recrystallization of the residue from EtOH gave tri-O-acetyl-β-DL-inosine (1.8 g.), m.p. 215~218°. *Anal.* Calcd. for $C_{16}H_{18}O_8N_4$: C, 48.73; H, 4.72; N, 14.21. Found: C, 47.98; H, 4.93; N, 13.80.

To the solution of 1.5 g. of tri-O-acetyl inosine in 70 ml. of abs. MeOH was added 100 mg. of $NaOCH_3$ and the mixture was refluxed for 20 min. After cooling, water was added and the aqueous solution was neutralized with IRC 50 (H^+) and evaporated to dryness *in vacuo*. To the residue was added 3 ml. of 88% alcohol and the solution was stored at 0° overnight. β-DL-Inosine (X) (0.66 g.) precipitated from the solution, m.p. 237~239° (decomp.). *Anal.* Calcd. for $C_{10}H_{12}O_5N_4$: C, 44.78; H, 4.51; N, 20.89. Found: C, 44.41; H, 4.65; N, 20.34.

β-DL-Isopropylidene Inosine (XI)——A solution of 0.85 g. of DL-inosine (X), 3.2 g. of bis(*p*-nitrophenyl) phosphate, 6.2 ml. of 2,3-dimethoxypropane in 70 ml. of acetone was stirred at room temperature for 6 hr. Evaporation of the solvent to dryness *in vacuo* gave red-brown gummy material which was eluted on IR-4B (OH^-) in 90% MeOH. The eluted fractions containing XI were combined and evaporated to dryness *in vacuo*. The residue was washed with water, and recrystallized from MeOH giving XI (0.49 g.), m.p. 255~258°. *Anal.* Calcd. for $C_{13}H_{16}O_5N_4$: C, 50.64; H, 5.23; N, 18.18. Found: C, 50.51; H, 5.09; N, 18.22.

β-DL-Inosine 5′-Phosphate (DL-IMP) (XII)——To a mixture of 0.41 g. of XI and 1.09 g. of bis(*p*-nitrophenyl)phosphate which had been dried under reduced pressure at 110°, 6 ml. of dioxane was added and the mixture was heated until a clear solution was obtained. After the solution was cooled at room temperature, 0.34 g. of ditolylcarbodiimide was added and the solution was stored over $CaCl_2$ in desiccator for 17 hr. The precipitate was filtered and washed with dioxane. The filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in dichloroethane and the pH of the solution was adjusted to 7.5 with aq. $KHCO_3$ solution. The organic layer was washed with water, dried and evaporated giving 9-[5-bis(*p*-nitrophenyl)phosphoryl-2,3-isopropylidene-β-DL-ribofuranosyl]hypoxanthine as amorphous solid (0.98 g.). This material was refluxed in 5 ml. of 1*N* LiOH for 2.5 hr. After cooling, insoluble material was separated by filtration and the filtrate was adjusted to pH 4.0 with dil. HCl and washed well with ether for removal of *p*-nitrophenol and then the solution was adjusted to pH 2.8 with dil. HCl. After aqueous solution was heated at 100° for 1.5 hr., *p*-nitrophenol deposited was removed by extraction with ether. To an aqueous solution was added aq. saturated $Ba(OH)_2$ solution to adjust pH to 9.0, and a small amount of insoluble material was removed by centrifugation. After three volumes of EtOH were added to the supernatant layer, the mixture was stored at 0° for 3 hr. The precipitates were collected by centrifugation and washed with 50% EtOH and 95% EtOH. The barium salt of XII thus obtained was decationized with Dowex 50 (H^+) in 5 ml. of water. The aqueous solution was evaporated to dryness *in vacuo* affording XII as powder. Compound XII was identified with an authentic natural inosine 5′-phosphate by UV, Rf value and electrophoresis.

The Resolution of DL-Adenosine——A) The enzymation of β-DL-adenosine (VII): 1) The method of experiment: Cells were harvested by centrifugation and washed with 20 ml. of water. Wet cells were then suspended into 10 ml. of 0.1*M* phosphated buffer, pH 7.0, containing 27 mg. of D- or DL-adenosine and incubated at 30° for 24 hr. to 120 hr. on a shaker. After removal of the cells by centrifugation the residual adenosine and its degradation products in the supernatant were identified on paper chromatography developed on Toyo Roshi No. 51 with water by ascending method at 10°. Detection was carried out with UV absorption after spraying ammonia-alcohol solution of eosine. For estimation of the nucleosides and other organic bases, UV absorption spectrum of the supernatant was measured.

2) Results of experiment: i) When D-adenosine was incubated with PS-264 at 30° for 24 hr., hypoxanthine was produced. However, the product was decomposed, leaving only a trace amount after 48 hr. (Fig. 1, 2 and 3).

ii) When β-DL-adenosine was incubated with PS-264 at 30° for 48 hr., inosine and adenosine were produced and after 120 hr. both products remained without decomposition (Fig. 1 and 4).

B) Separation of L-adenosine and L-inosine from incubation mixture: 1) Ion exchange resin chromatography method: The solution of DL-adenosine incubated with PS-264 for 72 hr. was adjusted to pH 10 with NH_4OH and passes through column with 25 ml. of Dowex 1 ($HCOO^-$). The aqueous ammonia (pH 10) was eluted and fractions were checked by UV spectrometer and fractions containing adenosine were combined and evaporated to dryness *in vacuo*. The residue was recrystallized twice from dil. EtOH giving β-L-adenosine, was dried by heating at 110° *in vacuo*, m.p. 230~231°. $[\alpha]_D^{27}$ +59.4° (c=1.2, H_2O). This β-L-adenosine was identified with authentic natural adenosine by UV and Rf value except $[\alpha]_D$.

After 200 ml. of 0.01*M* HCOOH-NH_4OH buffer solution (pH 6.8) was passed through the ion-exchange resin, adenosine was eluted with 0.01*M* HCOOH-NH_4OH buffer (pH 5.0). The fractions containing inosine (UV: $\lambda_{max}^{pH\,6.0}$ 249 mμ) were combined and evaporated to dryness *in vacuo*. The residue was recrystallized twice from dil. EtOH giving β-L-inosine, was dried by heating at 110° *in vacuo*, m.p. 218°. $[\alpha]_D^{27}$ +48.7° (c=1.2, H_2O). This β-L-inosine was identified with authentic natural inosine by UV and Rf value except $[\alpha]_D$.

2) Paper chromatography method: The incubated solution was concentrated to *ca.* 0.5 ml. *in vacuo*. The product was separated by chromatography on sheets of paper (Toyo Roshi No. 51, solvent: water, at 10° for 15 hr.). The cuttings of the paper containing both nucleosides detected by UV light were extracted with water and the extracts were evaporated to dryness *in vacuo* respectively. β-L-Adenosine was obtained from the lower part of paper and L-inosine from the upper.

The Resolution of DL-Adenosine 5′-Phosphate (IX)——A mixture of 70 mg. of β-DL-AMP (IX), 10 ml. of 0.1*M* ammonia-ammoniumchloride buffer (pH 8.5), 7 ml. of 0.005*M* $Mg(OAc)_2$ solution and 5. mg. of crude snake venom were incubated at 37~38° for 3 hr. The incubated solution was chromatographed on Dowex 1 ($HCOO^-$) (0.8×15 cm.) in distilled water. Fractions were checked by UV spectrometer and fractions containing adenosine were combined and evaporated to dryness *in vacuo*. The residue was recrystallized from dil. MeOH, and β-D-adenosine was obtained as needles (15 mg.), m.p. 227° (decomp.) (dried *in vacuo* at 110°). $[\alpha]_D^{26}$ −60° (c=0.5, H_2O).

This adenosine was identified with authentic natural adenosine by IR, and Rf value.

After 200 ml. of 0.01*M* HCOOH buffer solution was passed through the resin, L-AMP was eluted with 0.1*M* HCOOH buffer solution. Fractions were checked by UV spectrometer and fractions containing β-L-AMP was combined, neutralized with NH_4OH and concentrated to *ca.* 2 ml. *in vacuo*. To the solution was added saturated aq. $Ba(OH)_2$ solution to adjust pH to *ca.* 9.5 and a small amount of insoluble material was removed by centrifugation. After three volumes of EtOH were added to the supernatant, the mixture was stored at 0° for 3 hr. The precipitates were collected by centrifugation. The barium salt of AMP thus obtained was decationized with Dowex 50 (H^+) in 5 ml. of water to adjust pH to *ca.* 2.0 After the resin was removed, the aqueous solution was evaporated to dryness *in vacuo*. The residue was recrystallized from water-acetone giving β-L-AMP as needles. $[\alpha]_D^{26}$ +37° (c=1.28, pH 7). The β-L-AMP was characterized by its UV spectrum, paper chromatography, electrophoresis and $[\alpha]_D$.

The Resolution of β-DL-Inosine 5′-Phosphate (XII)——Treatment of ammonium salt of XII with 5′-nucleotides in the same manner as β-DL-AMP (IX) afforded β-L-IMP and β-D-inosine.

Ba-L-IMP $[\alpha]_D^{26}$ +18° (c=0.4, 2.5% HCl).

This IMP was identified with an authentic natural IMP by UV, Rf value and electrophoresis, except $[\alpha]_D$.

β-D-inosine $[\alpha]_D^{26}$ −45° (c=0.6, H_2O).

This inosine was identified with an authentic natural inosine by UV and Rf value.

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